

Treatment of feedyard dust containing endotoxin and its effect on weanling goats

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Accepted 26 July 2002

Abstract

Forty-two, mixed-sex, weanling goats were randomly allotted to six treatment groups: principal autoclaved dust ($n = 6$), control non-autoclaved dust ($n = 6$), principal dry-heat dust ($n = 6$), and control non-dry-heat dust ($n = 6$). Principals were treated with appropriate dust for one 4 h treatment in a closed tent. The data from the principal dust group ($n = 9$) and the control non-dust group ($n = 9$) were recorded after one 4 h dust treatment prior to the start of the present study. The endotoxin (ET) concentrations were determined for autoclaved dust ($13.3 \mu\text{g ET/g}$), dry-heated dust ($0.173 \mu\text{g ET/g}$), and non-treated dust $26.9 \mu\text{g ET/g}$. The tent aerosolized dust concentrations were: autoclaved dust $0.369 \text{ g}/(\text{m}^3 \text{ min})$ with $4.904 \mu\text{g ET}/(\text{m}^3 \text{ min})$; dry-heated dust $0.347 \text{ g}/(\text{m}^3 \text{ min})$ with $0.0015 \mu\text{g ET}/(\text{m}^3 \text{ min})$; and non-treated dust $0.539 \text{ g}/(\text{m}^3 \text{ min})$ with $4.904 \mu\text{g ET}/(\text{m}^3 \text{ min})$. These ET aerosol concentrations caused the autoclave dust goat group and the non-treated dust goat group to significantly increase their rectal temperatures at 4, and 8 h and total white blood cells (WBCs) increased at 12 and 24 h compared to their respective non-dust control groups. The dry-heat aerosol dust ET concentration in the tent did not induce an increased mean rectal temperature response or an increased mean total WBC count. Of the three principal dust products only the non-treated dust contained viable microbes.

Published by Elsevier Science B.V.

Keywords: Endotoxin; Feedyard dust; Aerosol application; Goat; Rectal temperature; Total white blood cell counts; Fibrinogen and haptoglobin concentration

1. Introduction

It has been shown that total suspended particles is a highly significant predictor of daily mortality

(Schwartz and Dockery, 1992). Airborne pollution affects human health which helps to set the primary standards governing air pollution. Air pollution also affects crops and natural ecosystems which help set the secondary standards of air pollution (Padgett and Richmond, 1983; Romieu, 1997). Smaller particles were indirectly related to higher respiratory death rates in the very young and the very old (Schwartz,

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1997). Mechanisms of non-biological ultrafine particle toxicity have been determined (Salvi and Holgate, 1999). Inhaled particles can carry toxic substances and free radicals on their surface which cause tissue damage when they are deposited at the gas exchange areas (alveoli) of the lungs. Oxidative damage occurs when free radicals, generated via transition metals associated with the particles, cause activation of alveolar macrophages. Activated macrophages release pro-inflammatory cytokines that amplify the inflammatory response. These inflammatory mediators enter the blood stream and affect blood platelets and clotting factors, thereby increasing plasma viscosity. This leads to cardiovascular dysfunction especially in patients with a compromised cardiovascular system.

Many agricultural practices produce particulate matter pollution in the atmosphere. For example, the preparation of fields for planting crops generates dust into the air (Gillette and Blifford, 1972). In addition, harvesting (Lighthart, 1982), and processing vegetable crops, such as flax, jute (Rylander and Morey, 1982), cotton (Pernis et al., 1961), hay (Siegel et al., 1991), wheat, oats, soybeans, corn, and the processing of grains, also leads to aerosolization of organic dust/endotoxin particulate matter (Clapp et al., 1993; Zuskin et al., 1989; Smid et al., 1992).

All Gram-negative bacteria produce endotoxins. The outer membrane of Gram-negative bacteria is composed of lipopolysaccharide molecules called endotoxin and the most toxic part of endotoxin is the lipid-A portion (Luderitz et al., 1978). Endotoxin is a relatively heat stable, biologically active material that profoundly affects both humoral and cell mediated immunity (Burrell, 1990; Morrison and Ulevitch, 1978). Complement and coagulation systems are affected by endotoxin and it interacts with many human cell types (Olenchock, 1997).

In order to determine the component most biologically active in feedyard dust, it was necessary to characterize the biological agents and the endotoxin contained in the organic dust, and the dust alone. Therefore, we compared autoclaved dust and oven dry-heated dust in weanling goats to determine if they produced a possible endotoxin fever response and leukocytosis following dust treatment, and to determine if viable microbes in the feedyard dust might contribute to these potential reactions.

The hypothesis was that autoclaved dust would preserve most of the endotoxin and totally inactivate all microbes; oven-heated dust would inactivate all endotoxin and microbes; and the non-treated feedyard dust would have intact endotoxin and viable microbes. These different dust treatments should therefore have different effects on the principal dust goat groups when compared to the controls.

The objectives of the study were to compare the goat treatment groups mean rectal temperature and total WBC counts, including the mean fibrinogen and haptoglobin concentration responses following one 4 h dust treatment with the appropriate dust for each of the three principal dust groups (dust, autoclaved dust and oven-heated dust) and their respective controls. All animal studies were approved by the regional animal care committee.

2. Materials and methods

2.1. Goats

Forty-two Spanish weanling goats of both sexes were housed in a three-sided barn. They were treated for internal helminth parasites (Ivomec MSD AGVET, Merck & Co. Inc., Rathway, NJ) and coccidia (Amprolium, MSD AGVET, Merck & Co. Inc., Rathway, NJ). Goats were in limit fed a commercial pelletized ration (44% grain concentrate, 20% alfalfa hay, 30% cottonseed hulls and meal, 5% molasses, Vitamins A and E and trace minerals) and watered free choice. The goats were randomly allotted to six groups, three dust principal groups with their appropriate three control groups. The groups were identified: dust group ($n = 9$) and their controls ($n = 9$); autoclave dust group ($n = 6$) and their controls ($n = 6$); and dry-heat dust group ($n = 6$) and their controls ($n = 6$). The goats were handled for 2 weeks prior to the start of the experiment so that they were accustomed to the feeding procedure, rectal temperature probe, and bleeding procedure.

The goats were housed in eight pens, three goats per pen (7420 cm²) and had access to feed (standard growing ration) and water ad libitum. Each dust treatment was 4 h. The rectal temperature and total WBC counts were measured following a time series relative to dust treatment: pre-dust treatment, (4 h dust treatment), 4, 8, 12, 24 and 48 h after dust treatment.

2.2. Clinical pathologic examinations

Total WBC counts and differential counts, and serum haptoglobin (Roy et al., 1969) and plasma fibrinogen concentrations (Millar et al., 1971) were assayed at the Texas Veterinary Medical Diagnostic Laboratory.

2.3. Tents

The semi-air-tight dust tent (183 cm wide \times 244 cm long \times 213 cm tall) was used to contain the administered dust and the goats. This has been previously described (Purdy et al., 2002a,b).

2.4. Feedyard dust preparation

Dried feedyard manure was removed from a working feedyard, processed to a fine dust (Purdy et al., 2002a,b) and stored in plastic barrels. This dust pool was divided into three parts; one part was left untreated; the second part was autoclaved; and the third part was dry-oven heated.

2.5. Autoclaved dust

The autoclaved feedyard dust was prepared by placing the dust in a pan at a depth of 1 cm and then autoclaving it for 15 min at 121 °C, under 6.9 kPa of pressure (Amsco Eagle Series Autoclave, 2021, Erie, PA). The dust was allowed to cool under vented conditions and then stored in a sealed zip-lock plastic bag.

2.6. Dry-oven-heated dust

The feedyard dust was placed in a pan at a depth of 1 cm in a dry-heat oven (Forma-Scientific Dryer-Oven #6097, Marietta, OH) at 180 °C for 6 h, after which it was allowed to cool, and then stored in a zip-lock plastic bag.

2.7. Microbial assay of dust

Both the autoclaved dust and the dry-heated dust were cultured in triplicate for bacteria on brain heart infusion (BHI) agar plates (Difco Lab., Detroit, MI) and for fungi on Malt agar and Littman oxgall agar

(LOA) (Difco Laboratories, Detroit, MI) plates. The BHI plates were incubated at 28 °C for 48 h, and the Malt and LOA plates were incubated for 7 days at 28 °C. The natural feedyard dust was assayed for bacteria and fungi and described in detail (Purdy et al., 2002a).

2.8. Dust aerosol technique used to challenge the goats

One thousand and five hundred grams of the appropriate prepared feedyard dust were placed in a hopper with a 0.6 cm auger in the bottom. The auger speed was set to deliver 950 g of dust over the 4 h dust treatment. The dust was augured into a metal funnel which led to a Jet Mill (Jet-O-Mizer, Fluid Energy Processing & Equipment Co., Hatfield, PA) under 138 kPa (Air pressure regulators, Wilkerson, Grainger, Amarillo, TX) which further pulverized it and separated larger particles which were lifted into a stainless steel trap (Trap, custom fabricated, In-Tox Products, Albuquerque, NM). An air vibrator (operated with 69 kPa of air) was attached to the outside of the hopper and touched the funnel which prevented dust build up in the funnel. The smaller particles from the jet mill were blown up a polyvinylchloride (PVC) pipe and out through PVC baffles located on the inside ceiling of the tent. Dust dissemination by the jet mill was augmented with air produced by a gasoline powered portable air compressor (Air compressor, Stewart-Warner, Johnson City, TN) and with air from a large blower motor (Portable Blower & Vacuum Motor, Industrial Type, 3 hp, velocity linear fpm 33,570, Grainger, Amarillo, TX).

2.9. Measurement of endotoxin/dust

Endotoxin/dust was measured using the kinetic chromogenic semi-quantitative *Limulus* amoebocyte lysate assay. This assay (Williams and Halsey, 1997) was non-reactive to glucans (BioWhittaker Inc., Walkersville, MD). Aliquots of the extracts were serially diluted in 10-fold increments with pyrogen free water. A 100 μ l aliquot of each dilution was mixed with 100 μ l of freshly prepared *Limulus* amoebocyte lysate containing chromogenic substrate in a pyrogen free microtiter plate (Dynatech Corp., Chantilly, VA) that was kept at 37 °C. Color development was monitored every 15 s with a microtiter plate-reading spectropho-

tometer (Dynatech MR5000, Dynatech Corp., Chantilly, VA). This instrument determines the time interval required to reach 0.03 absorbency and this was compared with an endotoxin standard curve covering the range of 5 ng/ml to 0.5 pg/ml. The standards were linear over a 5-log range of a log–log plot. Unknown samples were calculated by linear interpolation by the software. All dilutions were assayed in duplicate and a parallel dilution was spiked with 50 pg endotoxin to assess any enhancement or inhibition of activity by any of the extracted samples. Only those dilutions that did not exhibit enhancement or inhibition and which were parallel to the standard curve were used. Values were averaged on three different occasions. The coefficient of variations for these assays averaged 7.6%. To convert endotoxin activity to equivalent mass units of the EC6 reference standards (EC6 Reference Standards, US Pharmacopeia), a factor of 10 EU/ng was used. The analysis of endotoxin was conducted at the IBT Reference Lab., Lenexa, KS.

2.10. Statistical analysis

The mean results for measured variables were compared by analysis of variance using the General Linear Models procedures of SAS (1996). Means of rectal temperature, total WBC counts, absolute neutrophil and lymphocyte counts, and fibrinogen and haptoglobin concentrations were compared between principal and control groups over the experiment, and within any sample day period of time. Significant differences between treatment means were determined by Bonferroni's and Dunnett's adjusted paired *t*-test ($P \leq 0.05$) that allowed pairwise comparisons of treatment group means and control means within any sample collection day.

3. Results

The feedyard dust contained 26.9 $\mu\text{g ET/g}$, autoclaved dust contained 13.3 $\mu\text{g ET/g}$, and the dry-heated dust contained 0.173 $\mu\text{g ET/g}$. The autoclaved dust and the dry-heat dust was determined to be free of microbes. The dust treatment summary was outlined (Table 1).

The mean rectal temperature and clinical pathology parameters were compared among the three principal

dust treatment groups and their appropriate controls. The overall mean rectal temperature of the principal dust group ($n = 9$) was significantly different ($P \leq 0.018$) than the control group after one dust treatment. The mean rectal temperature of the principal dust group was significantly higher than its controls at 4 h ($P \leq 0.0361$) and 12 h ($P \leq 0.0080$) post-dust treatment (Fig. 1). The overall mean rectal temperature of the autoclaved dust group was significantly different ($P \leq 0.0002$) than the control group. The mean rectal temperature of the principal group was significantly higher than its control group at 8 h ($P \leq 0.0039$) and 12 h ($P \leq 0.0014$) post-dust treatment (Fig. 1). There was no significant difference between the mean rectal temperature of the principal dry-heated dust and its control group (Fig. 1).

The overall mean total WBC count of the principal dust group was significantly different ($P \leq 0.0001$) than that of its control group. The mean total WBC count of the principal dust group was significantly higher than the control group at 8 h ($P \leq 0.0116$) and at 12 h ($P \leq 0.0162$) (Fig. 2). The overall mean total WBC count of the principal autoclaved dust group was significantly different ($P \leq 0.0015$) than the control group. The mean total WBC count of the principal autoclaved dust group was significantly higher than the control group at 8 h ($P \leq 0.0180$) (Fig. 2). The overall mean total WBC count of the principal dry-heated dust was not significantly different than the control group (Fig. 2).

The overall mean absolute neutrophil count of the principal dust group was significantly different ($P \leq 0.0012$) than the control group. The mean absolute neutrophil count was significantly higher than the control group at 8 h ($P \leq 0.0001$) and 12 h ($P \leq 0.0019$) after the dust treatment. The overall mean absolute neutrophil count of the principal autoclaved dust group was significantly different ($P \leq 0.0001$) than the control group. The mean absolute neutrophil count of the principal autoclaved dust group was significantly higher at 8 h ($P \leq 0.0002$) and 12 h ($P \leq 0.0059$) than the control group. The mean absolute neutrophil count of the principal dry-heated dust was not significantly different than the control group (Fig. 3).

The overall absolute mean lymphocyte count of the principal dust group, principal autoclaved dust group and the dry-heat dust group were not significantly different than their respective controls. However, the

Table 1
Dust treatment summary

	Initial dust (g)	Hopper leftover (g)	Trap dust (g)	Dust in tent (g)	Dust in tent (g/(m ³ min))	Stage 1 (g)	Stage 2 (g)	Stage 3 (g)	Stage 4 (g)	Stage 5 (g)	Filter dust (g)	Dish dust total (g)	Tent floor dust (g/4.46 m ²)	Tent floor dust (g/m ²)
Dust	1500.0	264.6	5.2	1230.2	0.5388	0.3882	0.1788	0.1250	0.0446	0.0328	0.0141	7.4686	1095.18	245.59
Dry-heat dust	1500.0	239.2	418.9	841.9	0.3687	0.3331	0.1479	0.1342	0.0458	0.0372	0.0274	2.6884	394.22	88.40
Autoclave dust	1500.0	140.3	566.4	793.3	0.3474	0.2232	0.1131	0.1254	0.0444	0.0477	0.0184	3.2632	478.51	107.30
Mean (<i>n</i> = 3)	1500.0	214.7	330.2	955.2	0.4183	0.3148	0.1466	0.1282	0.0449	0.0392	0.0200	4.4734	655.97	147.10
S.E.	0.0	37.9	167.9	138.2	0.0605	0.0485	0.0190	0.0030	0.0004	0.0044	0.0039	1.5068	220.9487	49.55

One pool of dust was divided into three parts (part was autoclaved to preserve endotoxin and kill all microbes; dry-heated dust was heated in oven to destroy endotoxin and kill all microbes; non-treated dust preserved the endotoxin and microbes). The effects of each principal dust was compared in goats with their appropriate controls.

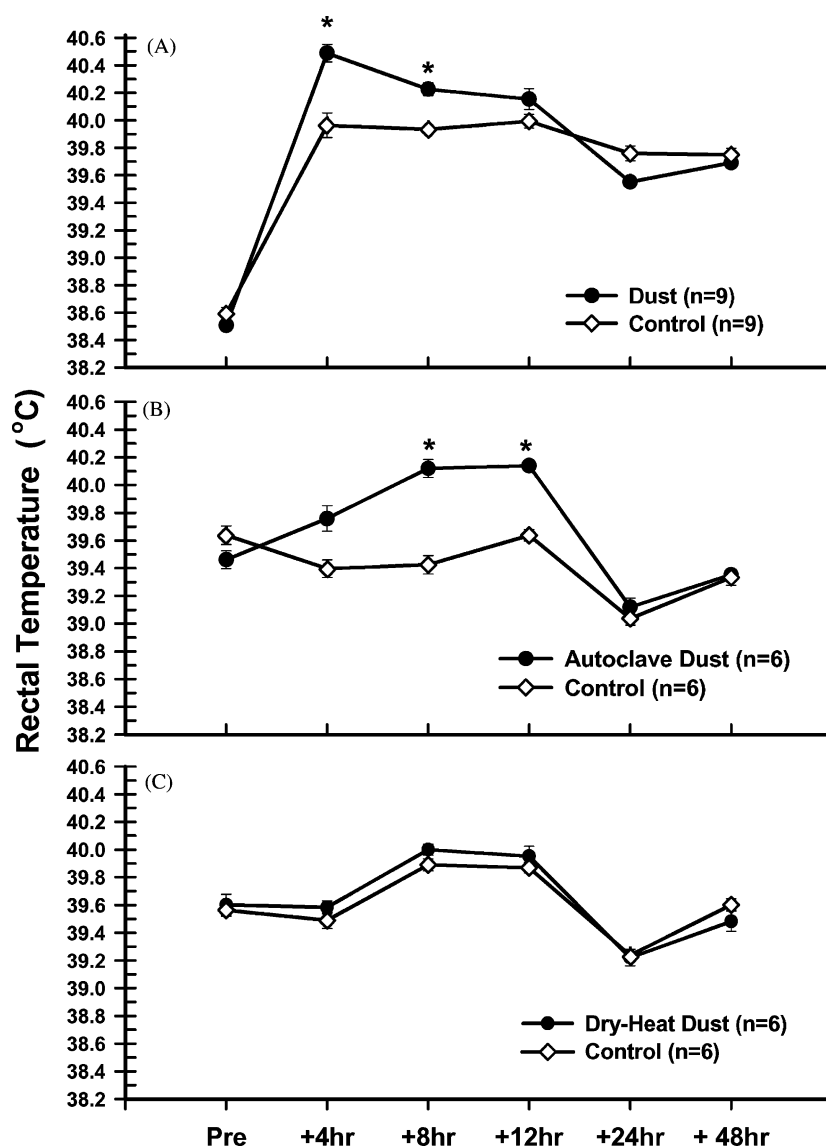


Fig. 1. The mean rectal temperature (°C) of the three principal dust groups (autoclaved dust preserved endotoxin and killed microbes; dry-heated dust destroyed endotoxin and microbes; untreated dust has endotoxin and viable microbes) compared to each of their controls after one 4 h dust treatment of appropriate dust. The asterisk indicates a significant mean rectal temperature increase in the principal dust group compared to the control group.

trend for mean absolute lymphocyte count of the principal dust group was decreased at 4 h and a decrease in lymphocytes was noted at 4 and 8 h for the principal autoclaved dust group. There was no decrease in absolute lymphocytes for the principal dry-heat dust group compared to controls (data not shown).

The overall mean fibrinogen concentration (mg/dl) of the principal dust group was significantly different ($P \leq 0.0281$) than the control group at 8 and 12 h. The mean fibrinogen concentration of the principal dust group was significantly higher at 4 h ($P \leq 0.0156$), 317 mg/dl and the fibrinogen concentration, although

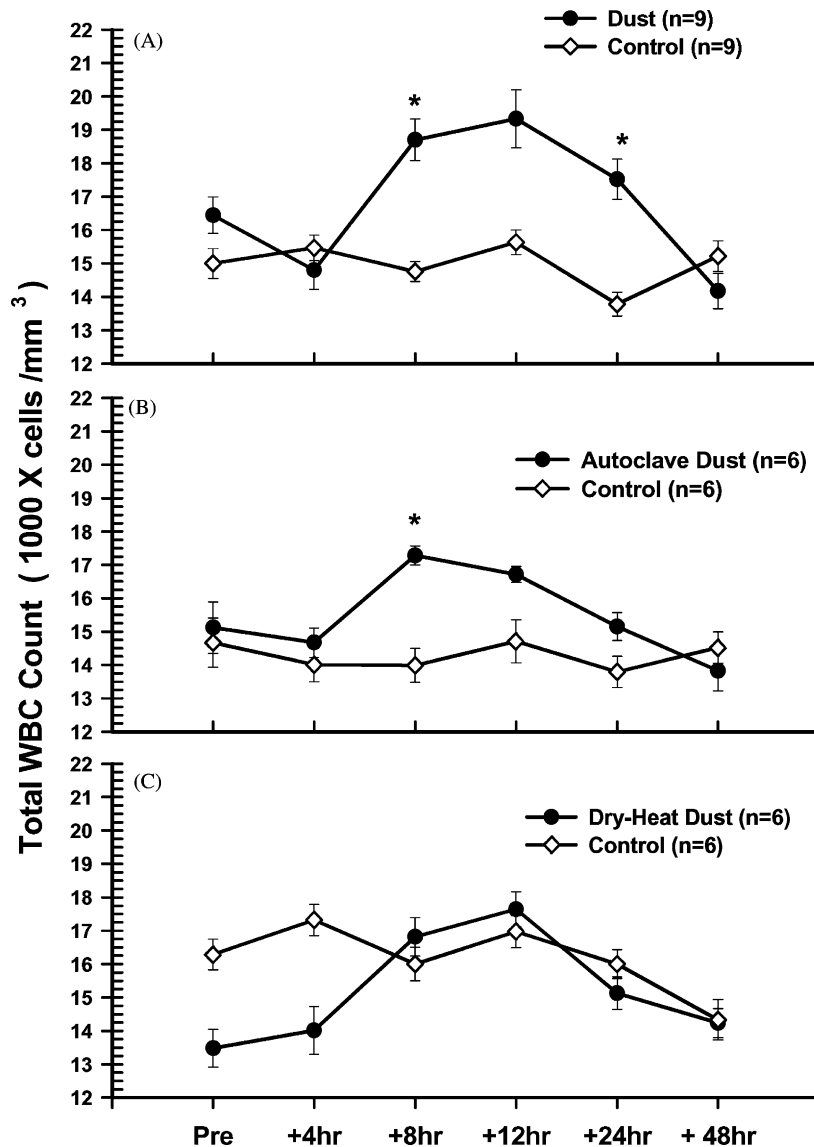


Fig. 2. The mean total white blood cell count (WBC) of the three principal dust groups (autoclaved dust preserved endotoxin and killed microbes; dry-heated dust destroyed endotoxin and microbes; dust has endotoxin and viable microbes) compared to each of their controls after one 4 h dust treatment of appropriate dust. The asterisk indicates a significant increase in mean total WBC of the principal dust group compared to the control group.

not significant, was higher at 8 h, 439 mg/dl and 12 h, 417 mg/dl than in the control group (4 h, 183 mg/dl; 8 h, 411 mg/dl; 12 h, 383 mg/dl). The overall mean fibrinogen concentration of the principal autoclaved dust was significantly different ($P \leq 0.0038$) than the control group. The mean fibrinogen concentration of the

principal autoclaved group, although not significantly higher, was higher at 8 h, 508 mg/dl; 12 h, 583 mg/dl; 24 h, 650 mg/dl; and 48 h, 600 mg/dl, compared to the control group (8 h, 408 mg/dl; 12 h, 525 mg/dl; 24 h, 567 mg/dl; and 48 h, 450 mg/dl). The mean fibrinogen concentration of the principal dry-heated dust group

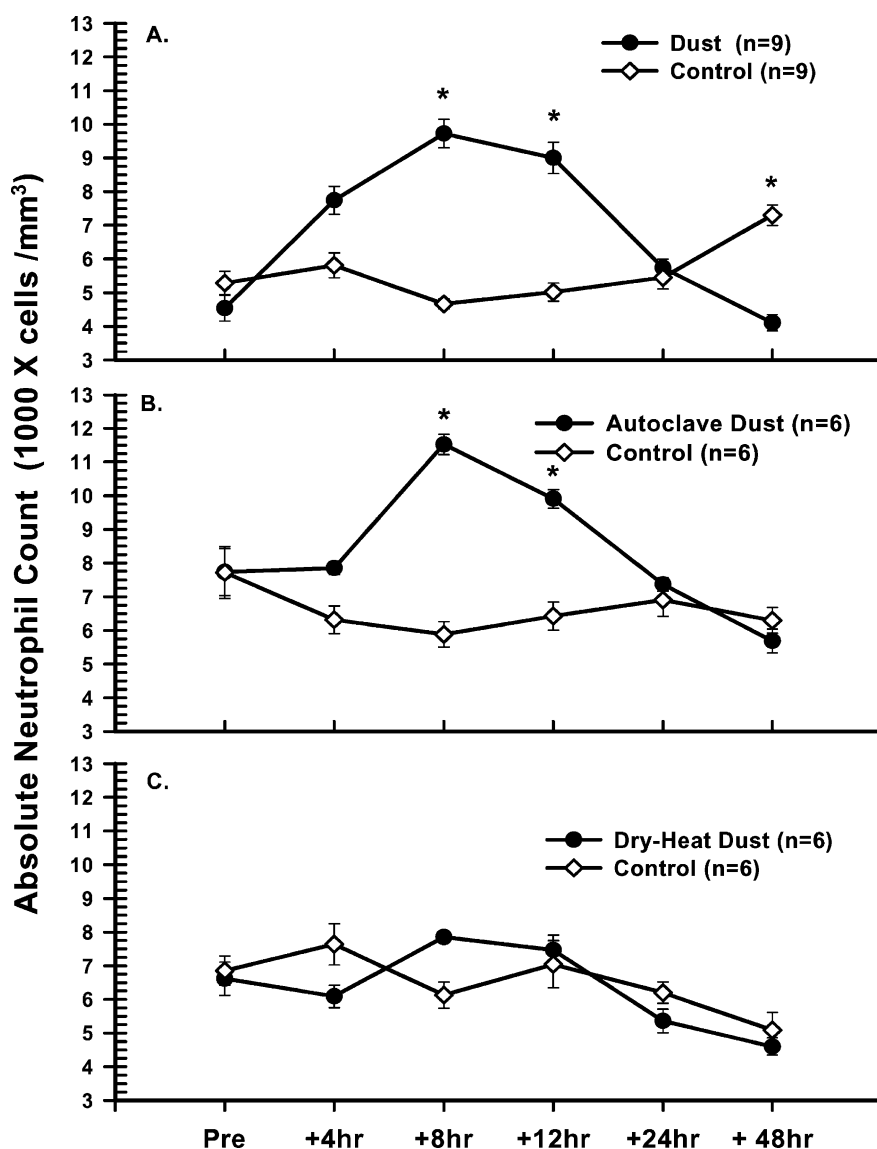


Fig. 3. The mean total absolute neutrophil count of the three principal dust groups (autoclaved dust preserved endotoxin and killed microbes; dry-heated dust destroyed endotoxin and microbes; dust has endotoxin and viable microbes) compared to each of their control groups after one 4 h dust treatment of appropriate dust. The asterisk indicates a significant increase in mean total absolute neutrophil count of the principal dust group compared to the control group.

was significantly different ($P \leq 0.0001$) at 8 and 24 h than the control group.

A haptoglobin response to dust was observed in only one principal dust goat. After one dust treatment, this goat demonstrated an increase in haptoglobin concentration from 0 to 25.6 mg/dl after 24 h. This increased

in haptoglobin concentration was observed on the first day of a 7-day dust treatment at 4 h, 18 mg/dl; 8 h, 61.2 mg/dl; 12 h, 80.4 mg/dl, next after the 7th day of dust treatment at 4 h, 204.7 mg/dl; 8 h, 212.5 mg/dl; 12 h, 206.1 mg/dl; and 48 h, 167.7 mg/dl. Haptoglobin responses were not observed in the autoclave dust

principals or dry-heated dust principals (data not shown).

4. Discussion

This series of experiments determined that the most significant biologically active component of feedyard dust under our test system was the endotoxin fraction, and not the culturable microbes, or the ultrafine dust which could carry other toxins and radicals. Because it was possible to measure the various fractions of the dust, we felt it was important to characterize the fractions and test each fraction in vivo to determine their individual effects.

It was determined that the untreated dust contained 0.539 g/(m³ min) of dust and 14.492 µg ET/(m³ min) which caused a significant rise in rectal temperature and total WBC counts in weanling goats when it was administered in a tent aerosol. The autoclaved dust contained 13.3 µg of ET per gram of dust. The autoclaved dust aerosol in the tent contained 0.369 g/(m³ min) of dust and 4.904 µg ET/(m³ min). The autoclaved dust aerosol contained 2.95 times less endotoxin than the dust aerosol. Both the untreated dust and autoclaved dust induced similar significant responses after 4 h treatments compared to their controls: rectal temperature increased over 4, 8, and 12 h, total WBC counts increased over 12–24 h, absolute neutrophils increased 8 and 12 h, and the trend for absolute lymphocytes was shown to decrease in 4–8 h.

The dry-heat treatment of dust almost totally destroyed the endotoxin content (0.173 µg ET/g dust). The dry-heated dust aerosol in the tent contained 0.347 g/(m³ min) of dust and 0.0015 µg ET/(m³ min). The goats treated with the dry-heated dust aerosol compared to the controls did not respond with increased rectal temperatures, increased total WBC, increased neutrophils, and decreased lymphocyte counts. The mean fibrinogen concentration (575 mg/dl) of the dry-heat principal group was significantly ($P \leq 0.0001$) increased at 24 h compared to the control group (308 mg/dl).

Several studies have examined the parenteral use of purified endotoxin in ruminants (Naylor and Kronfeld, 1986; Southorn and Thompson, 1986). However, there is a paucity of reports in which aerosolized endotoxin was used in other animals (Holst et al., 1994; Urbain

et al., 1999). Endotoxin has been used to induce mastitis in cows (Hopster et al., 1998). It is suspected that dust particles and meteorological parameters are in part involved with the induction of acute bovine respiratory disease in feedyards (MacVean et al., 1986).

There is a large body of published literature compiled on occupational endotoxin exposures in man (Edward, 1997; Rylander and Morey, 1982; Jagielo et al., 1996). It was concluded that endotoxin was the most biologically active part of organic dust inhaled by humans (Burrell, 1994). Humans have a very similar acute syndrome (Rylander et al., 1989) when they inhale endotoxin. They may develop a chronic syndrome, called byssinosis, first recognized in cotton textile mill workers over a hundred years ago in England (Schilling, 1956) and more recently (1960s) in cotton gin workers in the United States (McKerrow and Schilling, 1961; Bouhuys et al., 1967). The area with the highest risk (most dust/endotoxin) are called card rooms where the cotton is mechanically carded (brushed) prior to being spun into yarn (Castellan et al., 1987). This chronic lung disorder is caused by the inhalation of cotton dust which frequently contains *Enterobacter agglomerans* (Rylander et al., 1975) and fragments of the dead Gram-negative bacteria. The mechanism of action which induces byssinosis is controversial (Tockman and Baser, 1984). Nevertheless, in 1978, the Occupational Safety and Health Administration (OSHA) issued regulations that limit workers exposure to cotton dust (OSHA, 1978). This (OSHA) regulation was modified in 1985 (OSHA, 1985).

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